

***Echinoplaca infuscata* sp. nov. and new records of the genus *Echinoplaca* from Yunnan, China**

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ABSTRACT—Three *Echinoplaca* spp. are reported from China. The new species *Echinoplaca infuscata* is characterised by pale brown apothecia having different morphologies and colors at different growth stages, and (3–)5-septate ascospores. *Echinoplaca epiphylloides* and *E. intercedens* are reported as new from China. The macromorphology, micromorphology, secondary chemistry, ecology, and distribution ranges of these three species are presented and discussed. A key is provided to the *Echinoplaca* species known in China.

KEY WORDS—foliicolous lichen, *Ascomycota*, *Ostropales*, *Gomphillaceae*, taxonomy

Introduction

Echinoplaca Fée (*Ascomycota*, *Ostropales*, *Gomphillaceae*) was established by Fée (1825), with *E. epiphylla* Fée as the type species. The genus was previously placed in *Asterothyriaceae* (Santesson 1952), but it was later found that *Echinoplaca* and *Gomphillus* (*Gomphillaceae*) had homology in hyphophore morphology (Vězda & Poelt 1987). More than 50 *Echinoplaca* species are known worldwide (Lücking 2008, Kondratyuk & al. 2013, Wijayawardene & al. 2017).

In southern China, foliicolous lichens are abundant, but *Echinoplaca* lacks detailed study with only seven species reported in China: *Echinoplaca* cf. *epiphylla*, *E. handelii* (Zahlbr.) Lücking, *E. hispida* Sipman, *E. leucotrichoides* (Vain.) R. Sant., *E. fusconitida* Lücking, *E. pellicula* (Müll. Arg.) R. Sant., and *E.*

tetrapla (Zahlbr.) Lücking (Aptroot & al. 2003, Zahlbruckner 1930, Santesson 1952, Aptroot & Sparrius 2003). Here, we propose *Echinoplaca infuscata* as a new species; and *E. epiphylloides* and *E. intercedens* are reported as new to China. An identification key is provided for the known species of *Echinoplaca* in China.

Materials & methods

Specimens, morphology, and chemistry

The specimens were collected from Yunnan, China, and deposited in the Fungarium of College of Life Sciences, Liaocheng University, Liaocheng, Shandong, China (LCUF). Dissecting microscope (Olympus SZX16) and light microscope (Olympus BX53) were used for the macro- and micromorphological studies. Measurements were taken from mature vertical sections of fruit bodies mounted in water. Secondary metabolites were examined by color test (10% KOH, saturated solution NaClO and p-phenylenediamine dissolved in ethanol) and thin-layer chromatography (TLC) using solvent C (Culberson 1972, Culberson & Kristinsson 1970).

DNA extraction, PCR sequencing, and phylogenetic analysis

Genomic DNA was extracted from ascomata of the specimens using the REDExtract-N-Amp™ Plant PCR kits (sigma-aldrich, U.S. and Canada) according to the manufacturer's protocol. The LSU and mtSSU regions were amplified using the primer pair AL2R/LR6 (Lumbsch & Schmitt 2002, Lumbsch & al. 2004) and mrSSU1/mrSSU3R (Zoller 1999). The 50 µL PCR reaction system contained 2 µL each primer solution (10 µmol/L), 2.0 µL genomic DNA, 19 µL ddH₂O, and 25 µL 2×Taq PCR MasterMix (Tiangen, Beijing, China). Thermocycling conditions comprised initial denaturation at 95°C (5 min); 35 denaturation cycles at 94°C (45 s), annealing at 50°C (1 min), extension at 72°C (1.5 min); and a final extension at 72°C (10 min). The target product of PCR was assessed by electrophoresis on 1% agarose gels and sequenced by Biosune Inc. (Shanghai).

Contigs were assembled and edited using the program Geneious v6.1.2 (Biomatters Ltd., Auckland, NZ). The sequences from our new type specimen and GenBank data from 16 specimens of *Echinoplaca* spp. and an outgroup specimen of *Gyalectidium filicinum* (Amanda & al. 2022) were aligned using MAFFT v.7 (Katoh & Standley 2013). Multi-locus (mtSSU and LSU) phylogenetic analysis was performed. The combined analysis included 23 sequences (TABLE 1). The concatenated data matrix comprised 1479 nucleotide sites (LSU 552 bp and mtSSU 927 bp). Maximum likelihood (ML) and Bayesian inference (BI) were performed using the CIPRES Scientific gateway portal (<http://www.phylo.org/portal2/>) (Miller & al. 2010). Maximum likelihood bootstrapping analysis was performed, using the default parameters as implemented on the CIPRES, NSF XSEDE resource with bootstrap statistics calculated from 1000 bootstrap replicates (Stamatakis 2014). For the Bayesian analysis, the best substitution model was estimated using jModelTest 2.1.6 (Darriba & al. 2012). Based on the

results, we used GTR+I+G and GTR+G model. Bayesian analysis was performed using MrBayes 3.2.7a on CIPRES with 10 independent runs, searching for 10,000,000 generations with four independent chains and sampling every 1000th tree (Ronquist & Huelsenbeck 2003). After discarding the burn-in, the remaining 7500 trees of each run were pooled to calculate a 50% majority rule consensus tree. Generated phylogenetic tree was visualized and edited under Figtree v1.4.3.

Table 1. *Echinoplaca* and *Gyalectidium* sequences used in the analyses. Newly generated sequences are shown in bold.

SPECIES	SPECIMEN	COUNTRY	mtSSU	LSU
<i>E. campanulata</i>	Caceres & Lücking 168	Brazil	—	MZ851704
	Caceres & Lücking 171	Brazil	MZ827252	MZ851705
<i>E. diffluens</i>	Xavier-Leite 1901	Brazil	—	MZ851501
	Xavier-Leite 2001	Brazil	—	MZ851538
<i>E. epiphylla</i>	Caceres & Lücking 165a	Brazil	MZ827245	—
	Caceres & Lücking 165b	Brazil	MZ827246	—
<i>E. infuscata</i>	YN210763c	China	ON360972	ON117295
<i>E. leucotrichoides</i>	Xavier-Leite 1989	Brazil	MZ827184	MZ851534
	Xavier-Leite & Caceres 1734	Brazil	MZ827190	MZ851603
<i>E. lucernifera</i>	Lücking s.n., F	Costa Rica	AY341370	—
	Lücking 59c	Costa Rica	—	MZ851714
<i>E. marginata</i>	Xavier-Leite 1539	Brazil	—	MZ851489
	Xavier-Leite 1572b	Brazil	—	MZ851586
<i>E. pellicula</i>	Xavier-Leite 1502	Brazil	—	MZ851484
	Xavier-Leite 1560	Brazil	—	MZ851490
<i>E. tetrapla</i>	Xavier-Leite & al. 1422	Brazil	—	MZ851635
	Xavier-Leite & al. 1397a	Brazil	—	MZ851655
<i>G. filicinum</i>	Xavier-Leite 1571b	Brazil	MZ827278	MZ851584

Results

The phylogenetic trees obtained from maximum likelihood (ML) and Bayesian inference analysis (BI) exhibited the same topology. *Echinoplaca infuscata* obtained a high support value (97%/0.9935). The molecular phylogeny based on the mitochondrial large subunit ribosomal (LSU) and small subunit marker (mtSSU) of *Echinoplaca* exhibits a well-supported polyphyletic lineage (Amanda & al. 2022). *Echinoplaca infuscata* clustered with *E. pellicula*, but they can be distinguished easily in morphology.

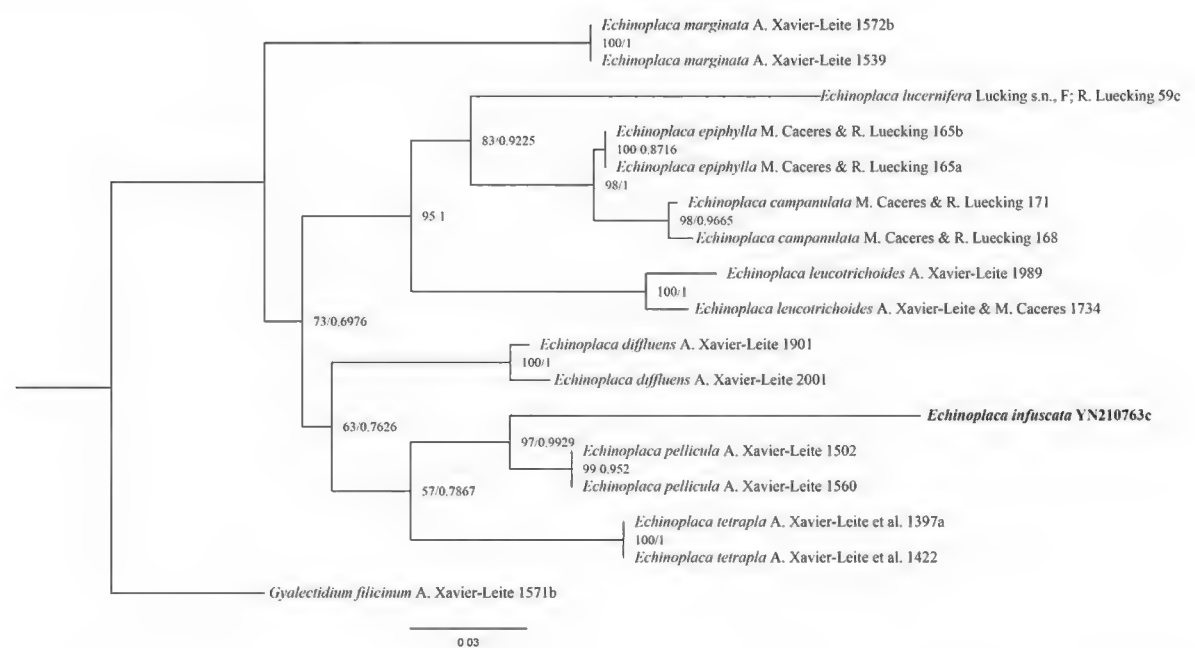


FIG. 1. The maximum likelihood (ML) phylogenetic tree generated from analysis of combined LSU and mtSSU of *Echinoplaca* spp., with *Gyalectidium filicinum* as outgroup. ML-bootstrap values/ Bayesian posterior probabilities are written next to nodes. Newly generated sequences are shown in bold.

Taxonomy

Echinoplaca infuscata M.L. Zhu & Z.F. Jia, **sp. nov.**

FIG. 2A–C

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Differs from *Echinoplaca tetrapla* by its brownish apothecia and (3–)5-septate ascospores.

TYPE: China. Yunnan Province, Mengla County, Rainforest Valley, Xishuangbanna National Park of Tropical Rainforests, 21.9156°N 101.1869°E, alt. 640 m, on leaves, 1 Jul. 2021, M.L. Zhu YN210763c (Holotype, LCUF; GenBank ON117295, ON360972).

ETYMOLOGY: The specific epithet *infuscata* refers to the brownish apothecia.

THALLUS foliicolous, crustose, continuous, pale green to green, with corticiform layer, finely white verrucose due to incrustation with calcium oxalate crystals, with scattered, sterile setae; verrucae 0.05–0.1 mm in diam., setae 0.5–1.0 mm long, white to pale brown. **PHOTOBIONT** a species of *Trebouxia*. **ASCOMATA** apothecia, adnate, spot-like, emarginate, rounded, 0.3–0.6 mm in diam.; disc plane, pale brown, often having different morphologies and colors at different growth stages, emarginate and darker brown initially, plane and pale brown when mature. **EXCIPULUM** colorless to pale brown, 20–55 µm wide; **EPITHECIUM** pale brown, 4–10 µm high; **HYMENIUM** colorless, 30–55 µm high; **HYPOTHECIUM** pale brown, 6–18 µm high. **ASCI** 4–8-spored, clavate, 26–44 × 9–24 µm, I–. **ASCOSPORES** ellipsoid, (3–)5-septate, with slight constrictions at

septa, $12\text{--}17 \times 5\text{--}7 \mu\text{m}$, about 2.5–3 times as long as broad. HYPHOPHORES not observed.

CHEMISTRY—Thallus K–, C–, KC–, P–. No substances detected by TLC.

ECOLOGY & DISTRIBUTION—On the leaves in tropical rainforest of Yunnan Province, China.

ADDITIONAL SPECIMENS EXAMINED: **CHINA. YUNNAN PROVINCE, Mengla County**, Rainforest Valley, Xishuangbanna National Park of Tropical Rainforests, 1 Jul. 2021 M.L. Zhu (LCUF YN210764b, YN210765a, YN210773a, YN210776a, YN210853b, YN210881a, YN210896).

COMMENTS—*Echinoplaca infuscata* is characterised by its pale green thallus, sterile setae sometimes present, white to pale brown; pale brown apothecia; and (3–)5-septate ascospores. *Echinoplaca tetrapla* and *E. intercedens* are closely related to *E. infuscata*, all of them having ascospores with 3(–5) septa, but *E. tetrapla* has greyish brown to brownish black, slightly shiny apothecia, *E. intercedens* often has dispersed thallus, and chocolate-brown apothecia (Lücking & al. 2001, Lücking 2008).

Echinoplaca epiphylloides Lücking, Fl. Neotrop., Monogr. 103: 476. 2008.

FIG. 2D–F

THALLUS foliicolous, crustose, continuous, pale greenish grey, irregular in outline, with cartilaginous, corticiform layer, finely verrucose and numerous sterile setae; verrucae 0.05–0.1 mm in diam., white; setae 0.6–1 mm long, white. PHOTOBIONT a species of *Trebouxia*. ASCOMATA apothecia, adnate, spot-like, emarginate, rounded, 0.4–0.6 mm in diam.; disc plane, pale yellow. EXCIPULUM colorless, 20–28 μm wide; EPITHECIUM pale brown, 5–10 μm high; HYMENIUM colorless, 40–50 μm high; HYPOTHECIUM colorless to pale brown, 6–12 μm high. ASCI 4–8-spored, clavate, $40\text{--}60 \times 10\text{--}15 \mu\text{m}$, I–. ASCOSPORES fusiform to oval, 5–7-septate, with constrictions at septa, $18\text{--}30 \times 4\text{--}8 \mu\text{m}$, about 3.5–4.5 times as long as broad. HYPHOPHORES not observed.

CHEMISTRY—Thallus K–, C–, KC–, P–. No substances detected by TLC.

SPECIMENS EXAMINED: **CHINA. YUNNAN PROVINCE, Mengla County**, Rainforest Valley, Xishuangbanna National Park of Tropical Rainforests, 1 Jul. 2021, M.L. Zhu (LCUF YN210763a, YN210771a, YN210816, YN210822b, YN210878a, YN210894, YN210987, YN211018b, YN211099).

DISTRIBUTION—Ecuador, Guyana, and Brazil (Lücking 2008).

COMMENTS—The species is reported from South America usually produces ascospores mainly with five septa, and filiform diahyphae with terminal segments narrowly fusiform (Lücking 2008). In Chinese material, we find

this species usually produces ascospores with 5–7 septa, and diahyphae were not found. *Echinoplaca pellicula* is similar to *E. epiphylloides* in the color of apothecia, but differs by its sparse sterile setae (Lücking 2008).

Echinoplaca intercedens Vězda, Acta Mus. Silesiae, Ser. A, 22: 83. 1973.

FIG. 2G–I

THALLUS foliicolous, crustose, continuous or disperse, pale gray-green to slightly white, with corticiform layer, fine verrucae and sterile setae; verrucae 0.05–0.15 μm in diam., setae pale brown, 0.1–0.3 mm long. PHOTOBIONT a species of *Trebouxia*. ASCOMATA apothecia, adnate, spot-like, emarginate, rounded, 0.4–0.7 mm in diam.; disc plane, brown to dark brown. EXCIPULUM colorless to pale brown, 15–30 μm wide; EPITHECIUM brown, 6–12 μm high; HYMENIUM colourless, 45–65 μm high; HYPOTHECIUM colorless, 7–15 μm high. ASCI 4(–8)-spored, clavate, 27–48 $\times$ 10–20 μm , I–. ASCOSPORES ovoid, 3–5-septate, with constrictions at septa, 11–18 $\times$ 4–6 μm , 2.5–3.5 times as long as broad. HYPHOPHORES not observed.

CHEMISTRY—Thallus K–, C–, KC–, P–. No substances detected by TLC.

SPECIMEN EXAMINED: **CHINA. YUNNAN PROVINCE, Mengla County**, Rainforest Valley, Xishuangbanna National Park of Tropical Rainforests, 1 Jul. 2021, M.L. Zhu (LCUF YN210829, YN210851b, YN210872a, YN210886, YN210988, YN210998b, YN211008, YN211019a, YN211068b, YN211069, YN211105b, YN211108a, YN211109).

DISTRIBUTION—U.S.A., Colombia, Ecuador, Brazil, and Bolivia (Lücking 2008).

COMMENTS—Our specimens conform to those described by Lücking (2008), which are characterised by its apothecia brown to dark brown and matt, and 3–5-septate ascospores. The most similar species is *E. triseptata*, which differs by having apothecia sordid to purplish brown, thallus usually with thin, white pruina, and ascospores 3-septate (Lücking 2008).

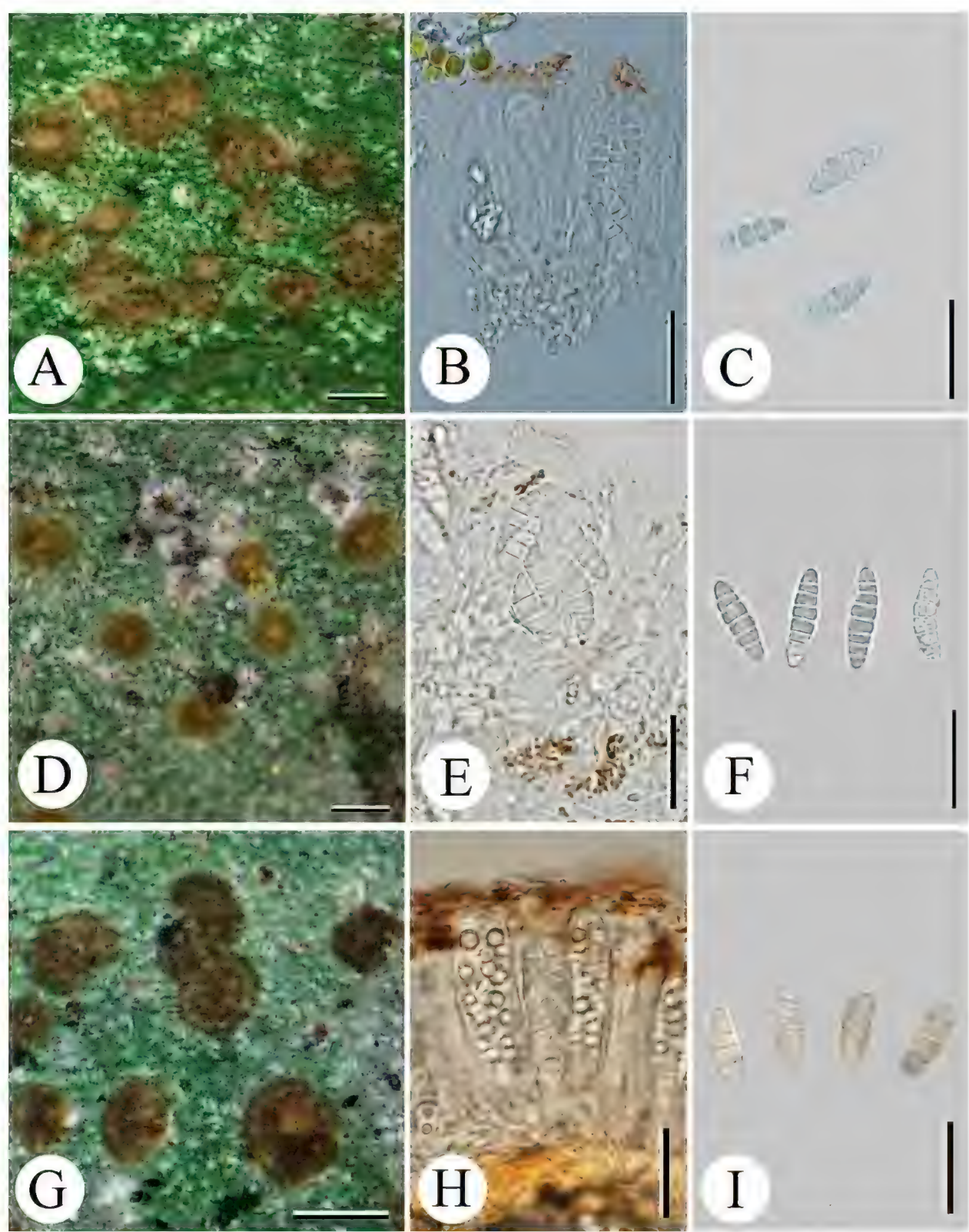


FIG. 2. Habitus, asci and ascospores of *Echinoplaca* spp. A–C: *Echinoplaca infuscata* (LUCF, Zhu YN210763c); D–F: *Echinoplaca epiphylloides* (LUCF, Zhu YN210987); G–I: *Echinoplaca intercedens* (LUCF, Zhu YN210829)

Key to the species of *Echinoplaca* known from China

- 1. Ascospores (sub-)muriform 2
- 1. Ascospores transversely septate 4
- 2. Ascospores no more than 30 µm long *E. handelii*
- 2. Ascospores more than 30 µm long 3
- 3. Apothecia dark greyish brown to blackish brown *E. fusconitida*
- 3. Apothecia pale yellow to orange *E. cf. epiphylla*
- 4. Ascospores 15–27-septate, 65–100 × 11–18 µm; apothecia 0.4–0.8 mm diam;
thallus finely verrucose *E. leucotrichoides*
- 4. Ascospores 3–7-septate 5
- 5. Thallus with sparse sterile setae; apothecia pale yellow to brown; ascospores
(3–)5(–7)-septate, 12–26 × 5–9 µm *E. pellicula*
- 5. Thallus with abundant sterile setae 6
- 6. Apothecia pale yellow to orange to brownish yellow 7
- 6. Apothecia brown 8
- 7. Apothecia pale yellow to orange; setae white *E. epiphylloides*
- 7. Apothecia orange to brownish yellow; setae pale brownish *E. hispida*
- 8. Apothecia pale brown; setae 0.5–1.0 mm long, white to pale brown; ascospores
12–17 × 5–7 µm *E. infuscata*
- 8. Apothecia dark brown or greyish brown 9
- 9. Setae 0.6–1.2 mm long; ascospores 11–20 × 4–6 µm *E. tetrapla*
- 9. Setae 0.1–0.3 mm long; ascospores 13–21 × 4–6 µm *E. intercedens*

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